Remarks/Arguments:

No amendments are made to the claims with this Response. Claims 1-30 are pending.

Applicants appreciate the Examiner's confirmation of the removal of the Li et al. reference from consideration.

II. Nonobviousness

The Examiner maintains the rejection under 35 U.S.C. §103(a) that the pending claims are unpatentable over Cho et al. (U.S. Patent No. 5,962,019) taken with Charman et al. (Pharmaceutical research, Vol. 9, No. 1, pp. 87-93, 1992) and Kovacs et al. (U.S. Patent No. 5,583,105).

A. A Lipophilic Compound does Not Necessarily form Spontaneous Emulsions

The Examiner maintains that the disclosure of Charman et al. disclosing the lipophilic compound of WIN 54954 which exhibits self-emulsification under gentle agitation teaches that self-emulsifying drug delivery systems are a convenient method of delivering hydrophobic drugs. From this teaching of convenience, the Examiner has apparently taken the position that it would have been obvious to provide any lipophilic active ingredient in the form of a spontaneous emulsion with the ingredients as claimed. For the reasons previously presented and set forth herein below, the applicants traverse this rejection.

The fact that WIN 54954 displays self-emulsifying characteristics and that it is lipophilic does not render obvious the use of any lipophilic compound, particularly cyclosporine as in the claimed invention to form spontaneous emulsions. This is because there no known set of criteria to predict whether a compound has self-emulsifying qualities. Self-emulsification is influenced by a variety of factors, including, polymer composition and molecular weight, polarity of organic solvent, composition of polymer blends, adsorption activity of drug and its ability to form complexes with polymers, and the presence of surfactants, to name a few. (See Babak et al, Colloid Aspects of Nano- and Micro- encapsulation of Bioactive Substances by Emulsification-Solvent Evaporation Method, a copy of which is enclosed).

In the present rejection, the Examiner is comparing cyclosporine with WIN 54954. Cyclosporine and WIN 54954 are very different compounds. The molecular weight for cyclosporine is 1202.61; whereas for WIN 54954, it is 411.32. Their corresponding molecular formulas are also drastically different: $C_{62}H_{111}N_{11}O_{12}$ and $C_{20}H_{24}Cl_2N_2O_3$ for cyclosporine and WIN 54954, respectively. Furthermore, WIN 54954 does not contain any peptide bonds and is

characterized by having oxazoline and isoxazole groups with an ether linkage. In contrast, cyclosporine contains amino acids linked by peptide bonds.

Because prediction of self-emulsifying characteristics can not be made and the drastic differences between cyclosporine and WIN 54954, the applicants submit that the fact that the two compounds are both lipophilic is insufficient to render claims 1-30 obvious.

B. Lack of a Motivation to Combine or Modify the Prior Art of Record

The Examiner asserts at page 6 of the Office Action that the claimed invention is made obvious by the combined teaching of the prior art since the instantly claimed invention falls within the scope of the prior art and method, which would have been *prima facia* obvious from the prior art disclosure to a person of ordinary skill in the art because such is held in a host of case law.

As discussed herein, the individual references do not stand for the proposition which the Examiner has asserted. References cannot be argued individually, but (as cited by the Examiner) rather must be taken for their collective teaching to one skilled in the art. Before this can happen, however, there must be some motivation to combine or modify the references. The Examiner has failed to set forth a motivation to combine or modify Cho et al. in view of Charman et al. or Charman et al. in view of Cho et al. Without such motivation, no *prima facia* case of obviousness has been made. MPEP § 2142.

C. The Examiner has Taken Contrary Positions

At page 4 of the Office Action, the Examiner states:

> Applicant alleges that Cho et al. does not teach a spontaneous formulation, let alone a spontaneous emulsion having the specified diameter of particles, or the specified concentrations, or the specified ratio of components.

The Examiner is misstating the applicants. The applicants were not submitting an argument, but paraphrasing what the Examiner had already acknowledged at page 4 of the 20 April 2004 Office Action, which is now quoted:

> The reference of Cho et al. differs from claims 1-30 in not teaching the formulation of a spontaneous emulsion and the specific concentration and ratios recited in the claims.

With this Office Action, the Examiner now asserts that Cho et al. suggests formation of a spontaneous emulsion because Cho et al. discloses aqueous colloidal dispersions of cyclosporine nanoparticles having good bioavailability. The Examiner further postulates that it is known in

the art that colloidal dispersion is by definition an emulsion (citation omitted). In conclusion, the Examiner states that Cho et al. "clearly suggest the formations of spontaneous emulsions."

The applicants respectfully request removal of this rejection for at least the following two reasons: (1) The Examiner is now taking a position on Cho et al. that is contrary to the Examiner's previous position regarding Cho et al. and (2) although a formulation that is a colloidal dispersion may be characterized as an emulsion, just because the formulation is a emulsion, does not means that was formed spontaneously.

(1) The Examiner's position regarding Cho et al. is critical to whether a *prima facia* case for obviousness has been properly made. In the present Office Action, the Examiner relies on Cho et al. as teaching formation of a spontaneous emulsion, which is contrary to a previous position. Without this teaching from Cho et al., the applicants submit that the claimed limitation of formation of a spontaneous emulsion and the specific concentration ratios are not met in the prior art.

(2) The Examiner overextends the teaching of Cho et al. As noted at page 4 of the specification, emulsions are dispersion of one immiscible phase in another, usually in the form of droplets. Emulsions are typically created by vigorous mechanical dispersion. Spontaneous emulsions form when added to an excess of water without the need for vigorous mechanical energy. The applicants assert that it is not known that a colloidal dispersion will instantaneously form a spontaneous emulsion when added to an excess of water without mechanical mixing. Thus, the Examiner's conclusion that because Cho et al. teaches a colloidal dispersion, it consequently teaches a spontaneous emulsion, is in error.

III. Objections to the Title, Specification, and Abstract

There is no recitation of the word "cyclosprine" in the title, specification, or abstract of the present application, contrary to the indication at page 7 of the Office Action. Applicants assume the Examiner takes issue with applicants' use of the alternative spelling of "cyclosporine" for "cyclosporin." As indicated in the attached copy of the Wikipedia free online encyclopedia, cyclosporine has a number of acceptable alternative spellings. Moreover, applicants assert that the use of "cyclosporine" is sufficiently clear to convey to one skilled in the art that which applicant's regard are their invention. Reconsideration of this objection is respectfully requested.

IV. Conclusion

In view of applicants' remarks set forth above, the Examiner has failed to set forth a *prima facia* case for obviousness: the Examiner has taken a contrary position in this Office Action compared to a subsequent Office Action; overreached when concluding that colloidal dispersions make obvious formation of spontaneous emulsions; failed to appreciate the difference between WIN 54954 and cyclosporine; and failed to provide sufficient motivation to combine or modify the prior art of record. Applicants respectfully request reconsideration and submit that the pending claims are in a condition for allowance. Early notification to that effect is earnestly solicited.

Respectfully submitted,

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April 18, 2005

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Colloid Aspects of Nano- and Micro-Encapsulation of Bioactive Substances by Emulsification-Solvent Evaporation Method

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Introduction

This presentation is done on the colloid aspects of one of the most wide-spreading method of the microencapsulation by simple oil/water or multiple water/oil/water emulsification followed by evaporation (extraction) of the organic solvent. The efficacy of this microencapsulation process is dependent on many formulation parameters which have an impact on the structure and the morphology of nano- and microparticles. These parameters are polymer composition and molecular weight, polarity of organic solvent, diffusion rate of the organic solvent from the emulsion droplets during evaporation (extraction) step, composition of polymeric blends used as wall forming material, adsorption activity of drug and their ability to form complexes with polymers, presence of surfactants in the formulation, viscosity, etc. Polymer crystallinity, polymer to core ratio, drug solubility, partition coefficient, etc. impact microparticle properties and drug release characteristics.

Proteins and enzymes are susceptible to denaturation, degradation, and conformational changes which may render them inactive. These conditions can be produced by adsorption at the solvent-water interface, complex formation with oppositely charged polymers and surfactants, mechanical processing etc. that may be encountered during microsphere production, storage and release. Innovative methods of protein protection during microencapsulation process must be developed [1].

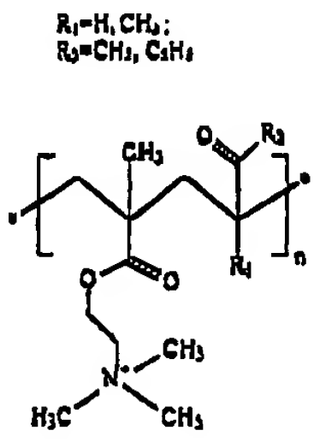
The release of drugs from the microcapsules in vivo may be realized by the diffusion through the porous walls or by the bio-destruction of the microcapsules. The first mechanism has the importance in the case of microparticles made from the non-biodegradable polymers, such as Eudragits RS and RL, whereas the second mechanism prevails in the case of biodegradable polymers, such as PCL and PLGA. The use of blends of both type of polymers permits coincide the both release mechanism and by this way to realise the control of the release rate of the drugs. In both release mechanisms, the internal and external morphologies of the polymeric micro-particles, particularly, closed and open porosity, multi-compartmentalbility, layered structure, etc. exerts a great influence on the prolonged release effect either by the influence on the diffusion rate of drugs through the porous matrices, or by the easy penetration of water inside the microparticle to produce swelling or hydrolysis effect. The morphology of the microparticles structure depends on the methods used to prepare microparticels and as well as on the physico-chemical parameters of the process.

The aim of this work is to bring more insight on the colloid aspects of the production of micro- and nanoparticles by emulsification/solvent evaporation (extraction) method and the possibility of tailoring the internal polymeric structure of microparticles by varying physico-chemical parameters.

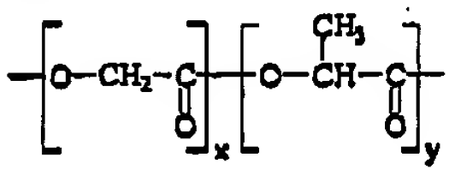
Materials. The biodegradable polymer poly[e-caprolactone] (*M* 10,000 Da) was purchased from Fluka (Steinheim, Germany). Polyvinyl alcohol (*M*w 20,000 Da, 80% hydrolyzed) chosen as surface active agents was supplied by Sigma (Steinheim, Germany). All compounds were of analytical grade purity. Eudragit RS and RL (acrylic polymers) were supplied by Rohm Pharma GmbH, Darmstadt, Germany. Fluorescein isothiocyanate (FITC) and rhodamine B isothiocyanate (RBITC) were purchased from Fluka (Deisenhofen, Germany).

Poly-e-caprocacton PCL

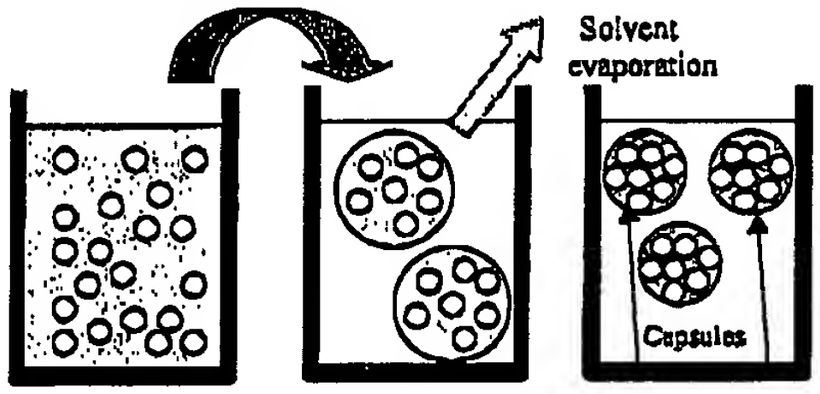
Eudragits RS and RL



PLA-GA



Preparation of microparticles. Microspheres based on Eudragit RS and blends of Eudragit RS :PCL 1:1 were prepared using an oil /water or water/oil/water emulsification-solvent evaporation (extraction) method. Total polymer amount of 250 mg was dissolved in 5 ml methylene chloride MC or ethyl acetate EA. This solution was poured into 1 l of 0,1 % w/w PVA and an oil /water-emulsion was formed by extensive stirring with a three-blade propeller at 1600 rev. /min during 2 hours.



The preparation of microparticles by the w/o/w-method was identical to the above described method with the exception that it was preceded by the formation of the first water/oil emulsion by the ultrasonication for 15 s of 1 ml of an aqueous solution of water soluble polymers into the 5 ml of polymer solution. In all cases, after decantation, the microparticles were filtered (HVLP filter, Millipore, pore size 0.45 μm), washed extensively with distilled water and dried overnight at room temperature.

Fluorescence labelling of Eudragits

Eudragit being a (meth)acrylic copolymer containing β-trimetyl-ammonium (up to 5 mol. %) and methoxy(ethoxy)carbonylic groups, was preliminary modified before being dye-labelled by means of reaction with 1,6-hexamethylen-dia-mine. Amino groups were introduced in Eudragit polymer in amount of 1,5-2,0 mol.% which did not alter physical and surface active properties of Eudragit. The fluorescent dyes, Fluorescein isothio-cyanate (Aldrich) and Lisamine rhodamine B sulfonyl chloride (Molecular Probes, Inc.) were used for modification of aminated Eudragit and preparation yellow FITC-Eudragit and carminic LRB-Eudragit, correspondingly. Efficiency of Eudragit labeling was determined photometrically using ε= 73000 L/Mcm at 490 nm for FITC-Eudragit (FITC-content:1.5 mol. %) and ε = 88000 L/Mcm at 570 nm for LRB-Eudragit (LRB-content : 2 mol.%) in dioxan: methanol (1:1 v/v) solution.

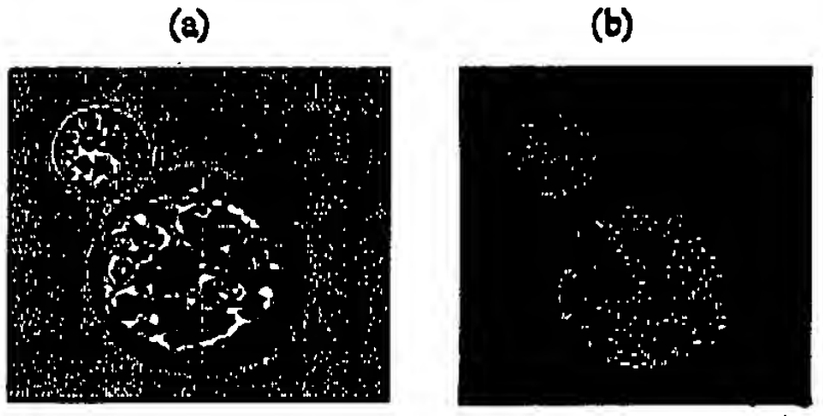


Fig. 1. Visualisation of microparicle containing chitosan made from the blend PCL and FITC-Eudragit RS (50:50) in MC by a light microscopy image (a) and by CLSM (b).

Fig. 2. SEM (a) and CLSM (b) images of microparticles obtained from individual Eudragit RS in MC

Results and discussion

As a rule, the internal structure of polymeric microparticles, obtained on the basis of only one polymer, presents dense and monolithic character (Fig.2), whereas the use of the blends of polymers leads to the phase segregation (Fig.4) and the formation of more complex ("brain-like") morphology (Fig. 3).

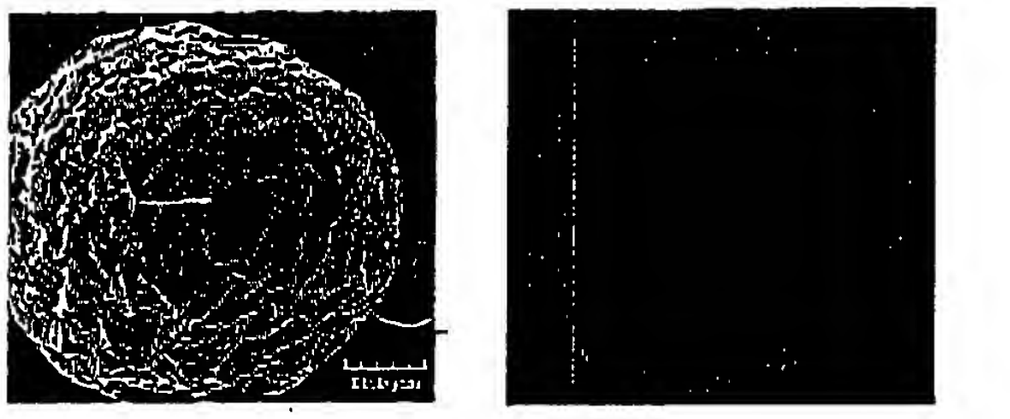


Fig. 3. SEM and CFM pictures of a microparticle on the basis of the blend Eudragit + PCL (1:1) obtained by evaporation of the organic solvent (methylene chloride).

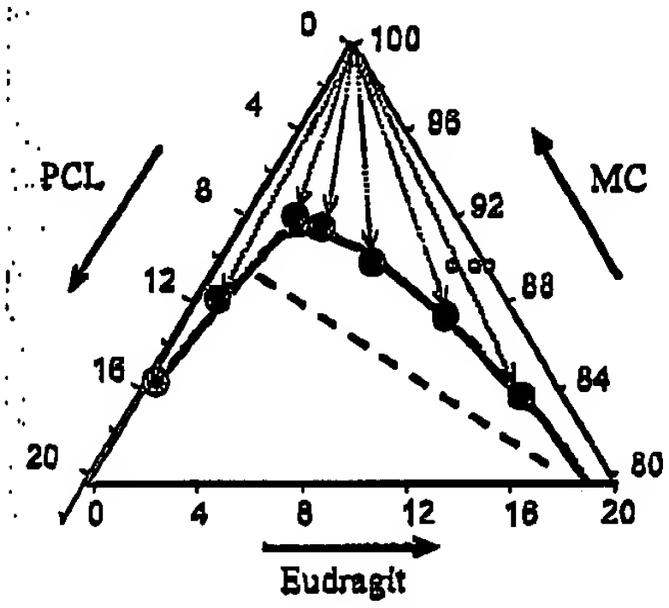


Fig. 4. Phase separation of the mixed methylene chloride solution for PCL and Eudragit RS.

The polymer PCL more than likely forms the matrix (Fig. 4) whereas the Eudragit clusters are the dispersed phase which fills this matrix.

Eudragit clusters localise near the surface of the microparticles, whereas the PCL is localised somewhere in the centre of the microparticles (Fig.3). This may be used to produce the layered structure by this diffusion enhanced phase separation.

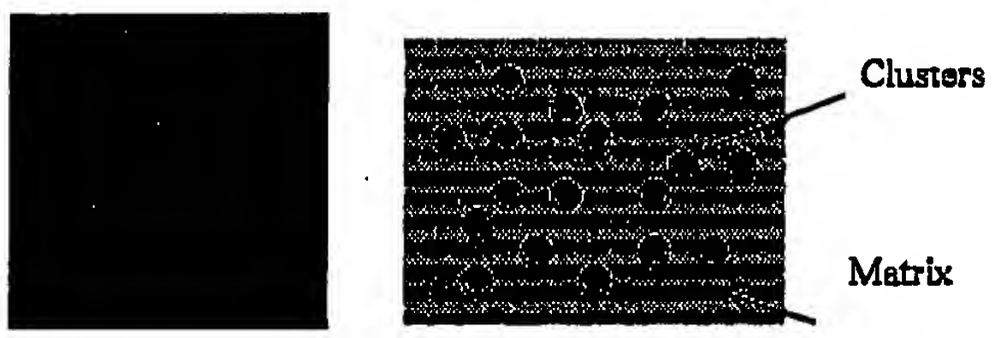


Fig. 4. Visualisation of the spinodal decomposition of the RBITC-Eudragit RS during the evaporation of the MC from the blend solution of Eudragit RS-PCL blend 1:1.

Thermomechanical tests prove (Fig.5) this internal structure of the polymeric blend. Really, the melting temperatures T_m for the PCL and the Eudragit RS are 60 °C and ~85 °C, respectively, whereas the temperature T_m for the PCL-Eudragit blend is equal to 60 °C, i.e. coincides with that of the pure PCL. So, the mechanical properties of the blend are governed by the properties of the PCL which forms the matrix.

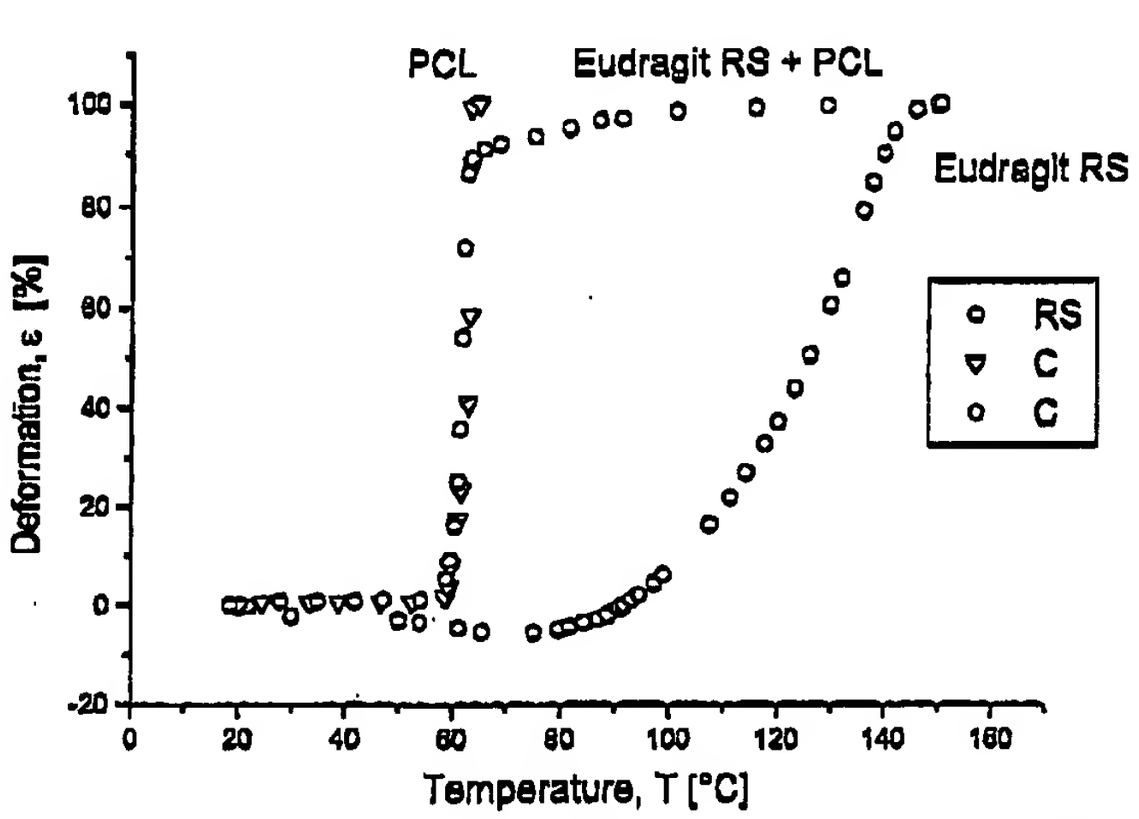


Fig. 5. Thermomechanical tests on the pure polymers and their 50/50 Blend.

Mechanism of the spatial self-organization of the internal nano-structure of microparticles obtained from the polymeric blends by the solvent evaporation method

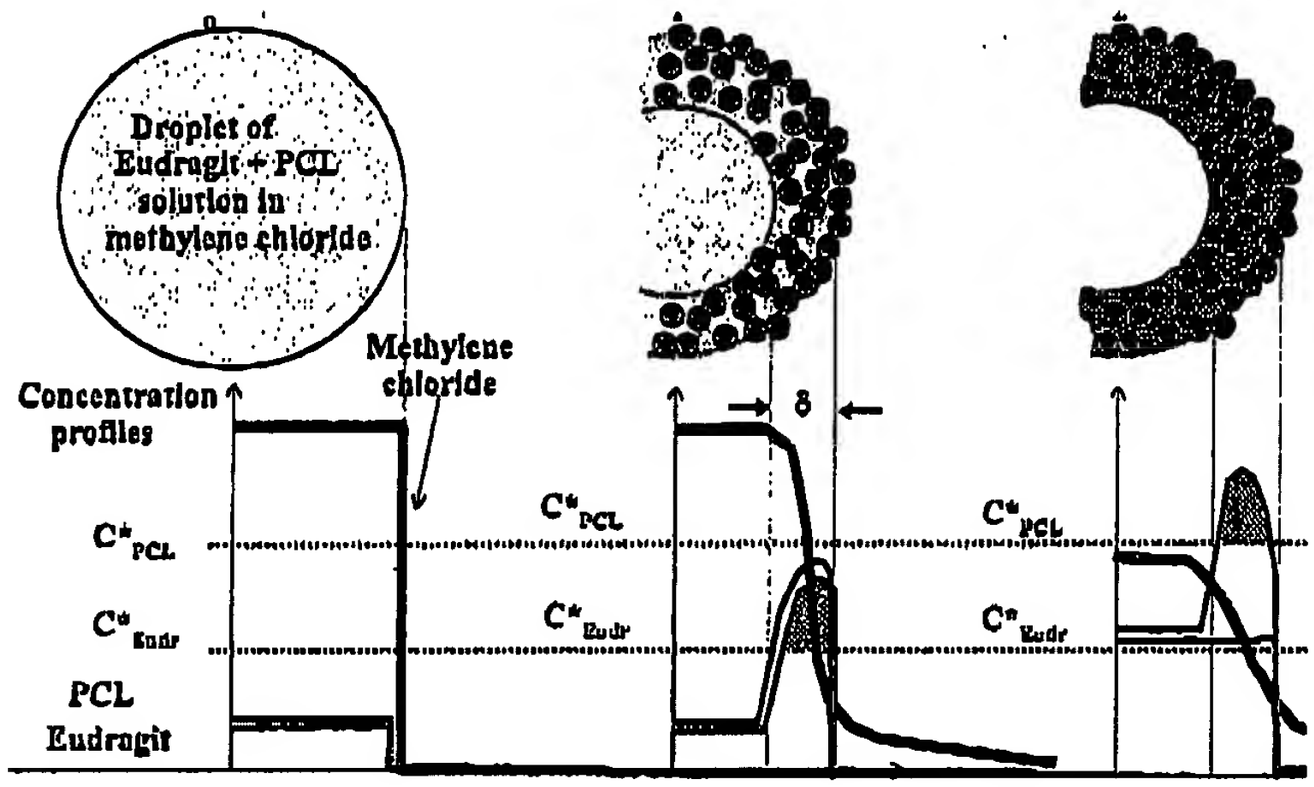


Fig. 6. Schematic representation of the formation of the structure of the microparticles by the evaporation of the solvent.

(a) At the time $t_0=0$ the concentrations of the organic solvent (MC), and polymers, Eudragit and PCL, in the droplet are characterized by the step-wise profiles; the concentrations of polymers are inferior to their solubility limits, C^*_{Eudr} and C^*_{PCL}, respectively.

(b) At the next stage, after evaporation of the MC into the water phase, its concentration profile in the near surface layer of thickness δ decreases; this leads to the eventual increase of the local concentrations of both polymers, Eudragit and PCL; the local concentration of the PCL does not exceed its solubility limit C^*_{PCL}, whereas the Eudragit does and consequently precipitates in the form of solid clusters in this layer.

(c) At the final stage of the evaporation, the local concentration of the PCL also exceeds its solubility limit C^*_{PCL}, that engenders its precipitation; the hole of the void may be formed if the contraction of the microparticle could not be possible in the course of the solidification (Fig. 1).

Conclusion

The presented preliminary results show the principal possibility to obtain microparticles and films with spatially organized nanostructure. It seems that the microparticles with layered structures of different polymer composition formed spontaneously may be interesting for the pharmaceutical applications. The more detailed report on the study of the effect of different physico-chemical parameters such as rate of solvent evaporation (extraction), type of polymers, size of microparticles and thickness of films, etc., will be done in the lecture.

Acknowledgment

The authors thank the COST 840 program for the invitation to the XIIth International Workshop on Bioencapsulation, Vitoria-Gasteiz, Spain.

References

1. Y.V.Chernysheva, V.G.Babak, N.R.Kildeeva, F. Boury, J.P.Benoit, N.Ubrich, Ph.Maincent. "Effect of the type of hydrophobic polymers on the size of nanoparticles obtained by emulsification-solvent evaporation". Mendeleev Communications, 2003, v.2, p. 65-68.

Ciclosporin

From Wikipedia, the free encyclopedia.

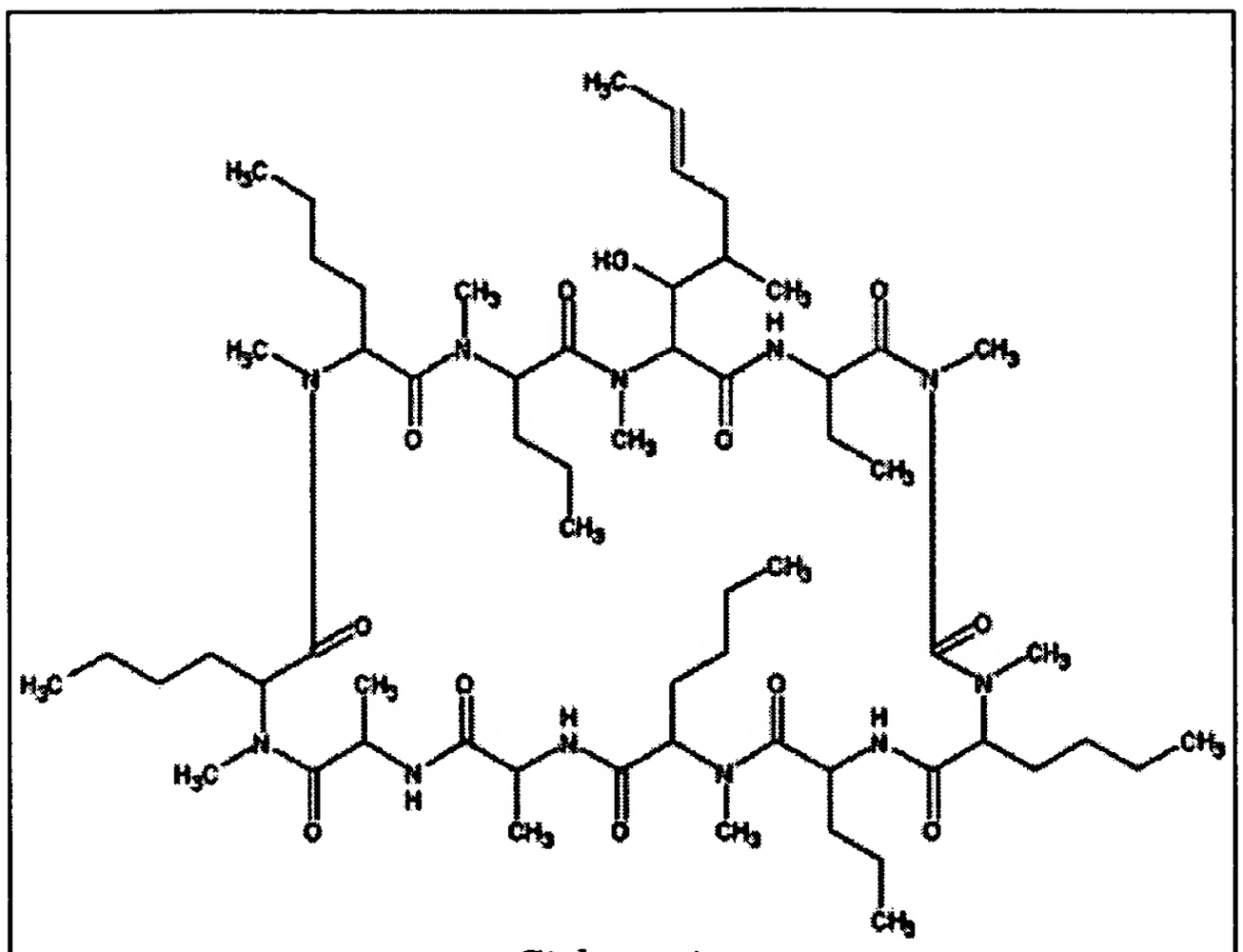
Ciclosporin (INN), **cyclosporine** or **cyclosporin** (former BAN), is an immunosuppressant drug. It is used post-allogenic organ transplant to reduce the activity of the patient's immune system and so the risk of organ rejection. It has been studied in transplants of skin, heart, kidney, lung, pancreas, bone marrow and small intestine. Cyclosporine is a cyclic nonribosomal peptide of 11 amino acids produced by the ascomycete fungus *Beauveria nive*.

The drug is sold by Novartis under the brand names **Sandimmune®** and **Neoral®**. Generic cyclosporine drugs have been produced by companies such as Sangstat, Abbott Laboratories and Gengraf. Since 2002 a topical emulsion of cyclosporine for treating keratoconjunctivitis sicca has been marketed under the trade name **Restasis®**. Annual sales of cyclosporine are around $1 billion.

Cyclosporine was discovered in 1970 in a Norwegian soil fungus by Jean F. Borel at Sandoz laboratories and it was approved for use in 1983.

Although the international noproprietary name is now *ciclosporin*, it is still referred to as *cyclosporine* in most scientific journals.

Contents



Ciclosporin

[R-[R,R*-(E)]]-cyclic(L-alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-L-leucyl-N -methyl-L-valyl-3-hydroxy-N,4-dimethyl-L-2-amino-6-octenoyl-L-α-amino-butyryl-N-methylglycyl-N-methyl-L-leucyl-L-valyl-N-methyl-L-leucyl)*

CAS number 59865-13-3	**ATC code** L04AA01
Chemical formula	$C_{62}H_{111}N_{11}O_{12}$
Molecular weight	1202.61
Bioavailability	variable
Metabolism	hepatic
Elimination half-life	variable
Excretion	biliary
Pregnancy category	C (USA) C (Aus)
Legal status	N/A
Routes of administration	?

Uses

Apart from in transplant medicine, cyclosporine is also used in psoriasis and infrequently in rheumatoid arthritis and related diseases, although it is only used in severe cases. It has been investigated for use in many other autoimmune disorders. It is often taken in conjunction with corticosteroids. More recently, cyclosporine has begun to be used to help treat patients suffering from ulcerative colitis with positive results.

Mode of action

Cyclosporine is thought to bind to the cytosolic protein cyclophilin (immunophilin) of immunocompetent lymphocytes, especially T-lymphocytes. This complex of cyclosporin and cyclophylin inhibits calcineurin, which under normal circumstances is responsible for activating the transcription of interleukin-2. It also inhibits lymphokine production and interleukin release and therefore leads to a reduced function of effector T-cells. It does not affect cytostatic activity.

Side-effects and interactions

Treatment has a number of potentially serious side effects and has adverse interactions with a wide variety of other drugs and other materials including grapefruit, although there have been studies to improve the blood level of cyclosporine with grapefruit juice. Side effects can include gum hyperplasia, convulsions, peptic ulcers, pancreatitis, fever, vomiting, diarrhea, confusion, breathing difficulties, numbness and tingling, pruritus, high blood pressure, kidney and liver disfunction, potassium retention and possibly hyperkalemia, hepatotoxicity, nephrotoxicity, and obviously an increased vulnerability to opportunistic fungal and viral infections.

See also

- tacrolimus.

External link

- PDF at Novartis (*http://www.pharma.us.novartis.com/product/pi/pdf/sandimmune.pdf*)

Retrieved from "http://en.wikipedia.org/wiki/Ciclosporin"

Categories: Immunosuppressive agents